

BASELINE SURVEY ON THE INSECTICIDE RESISTANCE PROFILE OF *ANOPHELES GAMBIAE* (DIPTERA: CULICIDAE) TO SELECTED INSECTICIDES IN IGBOKODA, SOUTHWESTERN-NIGERIA

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ABSTRACT

Malaria is a growing concern in African countries, including Nigeria. Among the multiple malaria control approaches, insecticides are of key importance, and this main practice is threatened by the emergence of resistance in malaria vectors. Studies on the susceptibility of *Anopheles* towards insecticides are lacking in Igobokoda Southwestern-Nigeria, and this study investigated the susceptibility status of *Anopheles gambiae* s.l using five selected insecticides including permethrin (0.75%), lambda cyhalothrin (0.05%), propoxur (1.0%), bendiocarb (0.1%) and pirimiphos-methyl (0.1%), which are frequently used as control against the vector population. Immature stages of *Anopheles* mosquitoes were collected using 350mL deep ladles and scooping spoons, and bred in the laboratory following the WHO standard protocols. Mosquitoes of age between 2 and 5 days were exposed to the selected insecticides using the WHO standard test procedure. Knock down time (KDT) for 50 and 95% was determined using probit analysis. Mortality of mosquitoes exposed to bendiocarb was 100%, followed by propoxur (98%), pirimiphos-methyl and permethrin (95%) and the lowest was observed for lambda-cyhalothrin (67%). Percentage knockdown was higher in bendiocarb compared to other insecticides and the difference was significant ($F= 5.2$, $P<0.0001$). KDT₅₀ and KDT₉₅ ranged between 12.07 to 54.12min and 28.18 to 91.38min, respectively, and was lowest for bendiocarb (12.07 and 28.18min, respectively). This study therefore imperatively suggests the continuous use of permethrin, adoption of bendiocarb or their combination for the treatment of long lasting bed nets as well as indoor coating of walls or spraying.

Keywords: *Anopheles gambiae*, Igobokoda, Insecticides, Ondo State, Susceptibility

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1. INTRODUCTION

Malaria is one of the major and growing challenge for human beings, transmitted by the infected bite of *Anopheles* mosquitoes. Malaria infection occurs after the transmission of one of the five Plasmodium species, leading to important clinical manifestations such as weakness, fast breathing and heartbeat, fever, chills amongst others, increasing morbidity and mortality rates in the population (Pluess et al. 2010; Agegnehu et al. 2018; Tesfa et al. 2018; Magago et al. 2019; Herekar et al. 2020). Malaria is almost present worldwide, with eleven countries marked as the world's most endemic regions and burdens reaching 70% (WHO 2020). Of these regions, ten were sub-Saharan African countries suffering about 50% of the total malaria disease, and Nigeria, the African countries with highest records of malaria disease in the world, and thus accounting for 27% of the disease prevalence (WHO 2020). Likewise, other African countries such as Democratic Republic of Congo recorded 11%, United Republic of Tanzania 5%, and Niger, Mozambique, and Burkina Faso 4%, respectively, while South-East Asia region equally suffers the malaria scourge (WHO 2020).

Mosquito control involving insecticide spraying of houses and insecticide treated nets are losing their effectiveness (Kelly-Hope et al. 2008; Tungu et al. 2021; McMillan et al. 2021), which have played a dominant role in reducing the transmission of malaria in the past (NMEP 2016) and six classes of insecticides have been classified for use (WHOPES 2007). Since the introduction of long-lasting insecticide treated nets (LLINs) in Nigeria in 2011, the total estimated

malaria disease has not exceeded 100 million cases (Nigeria Malaria Fact Sheet 2011), and population at risk which was reportedly 97% and 300,000 death tolls has decreased progressively with year.

Though in recent time, insecticide resistance has been the dominating factor challenging the Nigeria malaria elimination program (NMEP 2013). With increasing cases of resistance, there is need to reclassify and integrate control approaches rather than encouraging continuous use which could increase resistance levels (Beier 2008; Adolfi et al. 2019; Yunta et al. 2019). The factors responsible for the spread of insecticide resistance has been linked to mutations on target site and metabolic enzymes which probably cause the detoxification of insecticide and resistance in species (Balabanidou et al. 2019; Ingham et al. 2020). Intensifying the sustainable use of insecticide for malaria control with proper management is possible. Nevertheless, it is unclear whether climate change influenced the extent to which insecticide resistance spread or it is probable due to the differences in geographical regions. Global climate change could have directly or indirectly influenced mosquito abundance and distribution. Their preponderance in tropical Africa especially the sub-Saharan Africa than in other region (Cornel et al. 2018; Johnson et al. 2020) could be directly linked to temperature, precipitation, relative humidity and other environmental conditions (Mari and Jimenez-Peydro 2011; Gorsich et al. 2019), or indirectly related to artificial habitats that encourages water collection, adaptation and resistance profile (NMEP 2017).

Following the geographical spread of insecticide resistance, reclaimed action is required in malaria endemic regions where insecticide resistance is most especially occurring (Ononamadu et al. 2020; Chukwuekezie et al. 2020; Ojianwuna et al. 2021). These actions involving the adoption of insecticide mixtures as residual sprays and in treating bed nets could provide a newer dimension to malaria vector control. However, the challenges to this newer approach may relate to the knowledge and practice of insecticide application, as well as household perception of bed nets mass distributed. Currently, the focus of research is centred on determining the susceptibility of malaria vectors to recommended insecticides and a number of studies have been conducted in Nigeria (Djouaka et al. 2016; Chukwuekezie et al. 2020; Ononamadu et al. 2020). Residents in Igbokoda, Ondo State suffer several infections of public importance and malaria inclusive. Individuals use different kinds of chemicals include those selected in this study to ward off vectors. Insecticide bed nets (ITNs) used by residents are treated with permethrin and this study seeks to test their viability in susceptibility studies amongst other chemicals. Information on susceptibility of mosquitoes in Igbokoda, Ondo State is lacking, hence the need for this study to investigate the susceptibility of *Anopheles* mosquitoes to pyrethroid (lambda cyhalothrin and permethrin), carbamate (propoxur and bendiocarb) and organophosphate (pirimiphos-methyl) in Igbokoda, Ondo State, Nigeria. Specifically, the study monitored the susceptibility status of *Anopheles* species in four villages using the WHO standard test kit and determined the emergence of resistance in the population with regards the locations.

2. MATERIALS AND METHODS

2.1. Study Area

Igbokoda is located in Ilaje Local Government Area Ondo State, Nigeria. The villages selected for the study are indicated in Fig. 1, including Zion Igbokoda (Latitude: 7.2272710 and Longitude: 5.3276825), Kurugbene (6.2086503 and 4.7890091), Ago-Isobo (6.4562111 and 5.0200653) and Araromi Zion (7.0390534 and 5.6237984). Their selection as representative sample site for the study was done following the high reports of malaria by patients visiting the health facility in the area. Igbokoda experiences two seasons; rainy and dry. Rainy periods occur between April to November and dry between December to March. Temperature and relative humidity is $80\pm10^{\circ}\text{F}$ and $85\pm5\%$, respectively.

2.2. Mosquito Sampling and Breeding

Larvae of *Anopheles* mosquitoes were collected from potential breeding sites including ponds, ditches and puddles using 350mL deep ladles. The samples were collected in the early sunlit hours of the morning, during the raining season (May to July 2017). The wild collected specimens were transported in transparent plastic containers to the laboratory of Biological Sciences, Faculty of Science, Ondo State University of Science and Technology for culturing. The larvae were transferred to enamel pans, containing seasoned water, with the density of thousand larvae per litter and fed with a mixture of 10g yeast to a stick of cabin biscuit, netted with thin muslin cloth to prevent the escape after emergence. Upon adult eclosion, mosquitoes were transferred to the holding cages (30 cm \times 30 cm \times 30cm) and were fed on 10% of sucrose solution. *Anopheles* mosquitoes were distinguished from other species in the holding cage using their resting positions, pattern of the antenna among other body parts using standard identification key described by Gillet (1972) and Gillies and Coetzee (1987). Using an aspirator, other mosquito species were removed from collection, and males were sorted from females. The females were fed with 10% sugar solution twice *ad libitum*. Collection was maintained in climate-controlled standard laboratory with a temperature of $28\pm3^{\circ}\text{C}$ and a relative humidity of $80\pm5\%$.

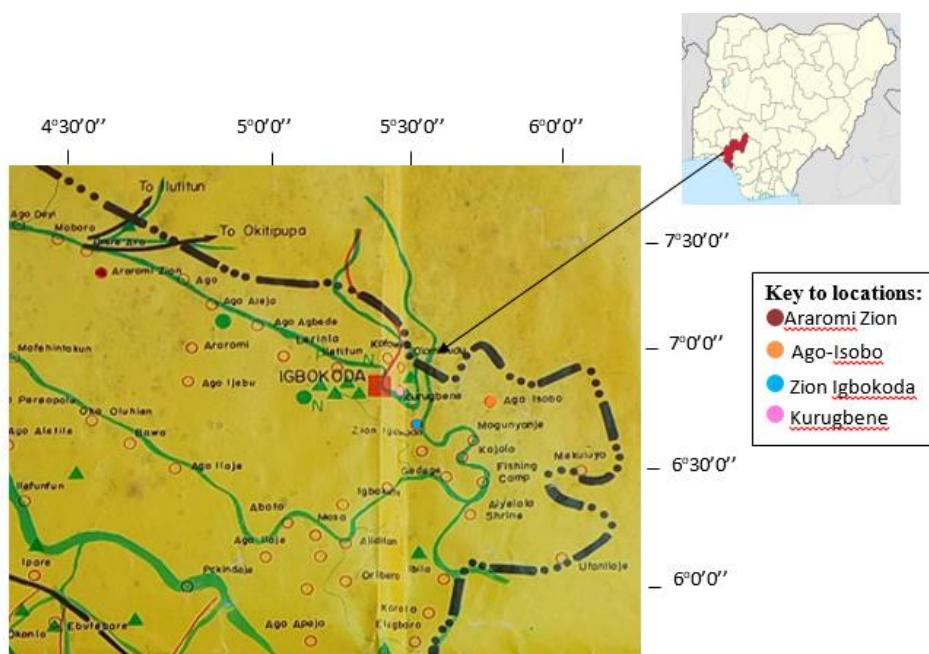


Fig. 1: Map of Nigeria showing the study locations in Igbokoda.

2.3. Insecticide Resistance Test

WHO test procedure described in WHO (2020) was adopted for this study. Insecticide impregnated papers with different diagnostic doses were obtained from the WHO collaboration center at the Nigerian Institute of Medical Research (MIMR), Yaba, Lagos State, Nigeria. Lambda cyhalothrin (0.05%), permethrin (0.75%), propoxur (0.1%), bendiocarb (0.1%) and pirimiphos-methyl (0.25%) were tested. Twenty-five unfed females, aged between 2-5 days were exposed to insecticide in each test tube internally lined with impregnated papers, and were replicated in four for each insecticide, whereas the 2 control tubes were coated with filter papers containing olive and silicone oil for each time of testing. Minimum of 100 females were exposed to each insecticide concentration. Knock down records were taken for 10, 15, 20, 30, 40, 50 and 60mins. At the completion of the experiment, mosquitoes were gently transferred into the holding tubes, provided with adult diet via cotton wool.

2.4. Mosquito Analysis

At the completion of the experiment, mosquitoes susceptible to the insecticide were confirmed by identifying again under an electrified dissecting microscope using standard manual by Gillet (1972) and Gillies and Coetzee (1987) and the resistant species were killed in a killing jar with the help of ethyl acetate soaked in cotton wool before identification. After identification, the female *Anopheles* mosquitoes were preserved in an Eppendorf tube containing silica gel following WHO guidelines for species identification.

2.5. Data Analysis

Data were entered into MS Excel, (version 2013), checked for typographical errors and corrected. Abbott formula (Abbott 1925) was used to correct the mortality where applicable. Knockdown of mosquitoes over time was presented in percentages. Line chart was used in presentation of results. Mortality of ≥ 98 shows susceptibility, 90 -97% shows suspected resistance, ≤ 90 shows resistance. Two-way ANOVA was used to determine the significance within insecticides and time. Significance was set at $\alpha=0.05$. KDT₅₀ and KDT₉₅ were computed and modelled using the Probit analysis and Pearson goodness of fit, respectively.

3. RESULTS

3.1. Mortality of *Anopheles* Mosquitoes Over Insecticides

The mortality of *Anopheles* mosquitoes exposed to lambda cyhalothrin, permethrin, propoxur, bendiocarb, and pirimiphos-methyl in Igbokoda, Ondo State, Nigeria is presented in Table 1. Bendiocarb exposure had the highest mortality followed by Propoxur. However, suspected resistance was observed with Permethrin and Pirimiphos-methyl exposures (95%, respectively), while specimens exposed to Lambda-cyhalothrin were resistant (67%).

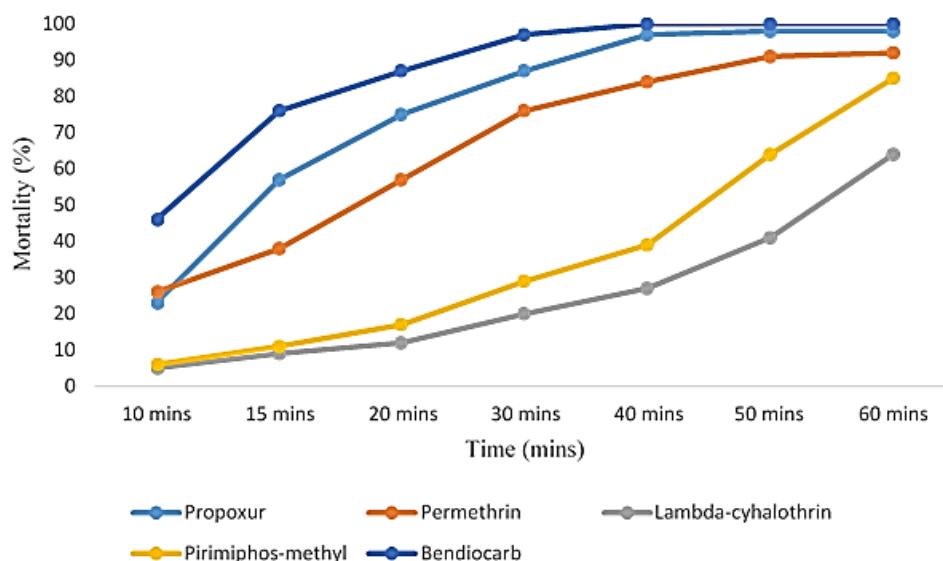
Table 1: Mortality of *Anopheles* mosquitoes exposed to five selected insecticides in Igbokoda, Ondo State, Nigeria

Insecticide	% Mortality (24hrs)	Resistance status
Permethrin	95.00	Suspected resistance
Lambda-cyhalothrin	67.00	Resistance
Propoxur	98.00	Susceptible
Bendiocarb	100.00	Susceptible
Pirimiphos-methyl	95.00	Suspected resistance

Note: ≥98 shows susceptibility, 90-97% shows suspected resistance, ≤90 shows resistance.

3.2. Knockdown of *Anopheles*

Knock down of mosquitoes increased with time in all the exposures. Over the 60min exposure, 100% knockdown was observed with bendiocarb from 40 to 60min. Similarly, knock down recorded over 90% in mosquitoes exposed to propoxur from 40 to 60min. Permethrin exposure recorded over 80% knockdown while, this time was observably low in lambda-cyhalothrin and pirimiphos-methyl. However, at 60min, both insecticides recorded 64 and 85% knockdown respectively (Fig. 2). The differences between knock down of *Anopheles* mosquitoes exposed to the five insecticides were significant ($F_{(insecticides)} = 44.06$, $P < 0.0001$; $F_{(Time)} = 24.80$, $P < 0.0001$).


Fig. 2: Knockdown of *Anopheles gambiae* s.l from Igbokoda, Ondo State, Nigeria to propoxur, permethrin, lambda-cyhalothrin, pirimiphos-methyl and bendiocarb for 60 minutes.

3.3. Knock-down Over Time

The mosquitoes exposed to the five insecticides showed remarkable knock down time of 50 and 95%. It was observed that KDT₅₀ values ranged from 12.07 to 54.12 minutes while the KDT₉₅ values ranged from 28.18 to 91.38 minutes. The model of the exposure to various insecticides followed a well fitted curve according to the Pearson goodness of fit (Table 2).

4. DISCUSSION

The study has provided an update on the current level of resistance profile of female *Anopheles* mosquitoes from Igbokoda, Ondo State, Nigeria to lambda-cyhalothrin, permethrin, propoxur, bendiocarb, and pirimiphos-methyl. The WHO bioassay results indicated a complete mortality with bendiocarb, mosquito susceptibility with propoxur, suspected resistance with permethrin and pirimiphos methyl and resistance with Lambda-cyhalothrin. Similar patterns of mortality were recorded for permethrin and pirimiphos-methyl. Insecticide treated nets and indoor coating of walls with insecticides are two most common and acceptable methods for vector control (Hougaard et al. 2002; Mabaso et al. 2004; N'Guessan et al. 2007). The ability of various insecticides selected for this study to elicit mosquito susceptibility except permethrin and pirimiphos-methyl with suspected resistance and only lambda-cyhalothrin recording resistance showed that situation of insecticide resistance is not worsen in the study locations. This is in agreement with earlier studies in the geographical zone within Nigeria (Chukwuekezie et al. 2020; Ononamadu et al.

2020). Similarly, the complete mortality observed for exposure to bendiocarb in this study corroborate with the study of Abdalla et al. (2008).

Table 2: Knock down over time of *Anopheles* mosquitoes to five insecticides in Igbokoda, Ondo State, Nigeria

Treatments	Regression line	Pearson χ^2 goodness of fit	KDT ₅₀ (50% CI)	KDT ₉₅ (95% CI)
Permethrin	Y= 4.21X - 5.34	259.67	18.26 (16.21-22.17)	71.38 (62.08-90.91)
Lambda-cyhalothrin	Y= 3.51X - 4.75	162.29	54.12 (40.23- 49.21)	82.06 (73.21-114.25)
Propoxur	Y= 3.25X - 4.46	135.18	13.81 (11.02- 16.05)	35.82 (29.51- 42.70)
Pirimiphos-methyl	Y= 3.24X - 4.71	167.42	46.11 (43.90- 59.13)	73.18 (68.07- 97.10)
Bendiocarb	Y= 4.10X - 4.53	139.67	12.07 (10.19-15.11)	28.18 (25.02-35.14)

Note: Total number of mosquitoes exposed (n=100 in each treatment); 50% and 95% Knock down time, KDT₅₀ and KDT₉₅, are in minutes; 95% confidence interval; P>0.05 suggests a well-fitting model, P<0.05 suggests an invalid model population.

The spread of insecticide resistance depends on the extent to which insecticides are used with time or intensified in inappropriate quantities. Igbokoda is a fishing village and suffers several diseases caused by insects. The use of germalin 20 for fishing has been observed previously by residents in the area and the likelihood of using several other chemicals in insect control and agricultural purpose is possible. This may have resulted in the suspected resistance and resistance in the respective insecticides reported in this study which corresponds to the reports of Knox et al. (2014) and Diabate et al. (2002).

Insecticide resistance in *Anopheles* has been reported in Nigeria (Awolola et al. 2002; Awolola et al. 2007; Okorie et al. 2015), in Benin republic (Djogbénou et al. 2009; Djègbé et al. 2011), in Zimbabwe (Munhenga et al. 2008) and many other tropical countries, where mosquito biting activities are high. Successful determination of resistance status in malaria endemic areas is important to inform policy makers on which insecticide is more effective to guide the necessary vector control actions. This study further confirms the existing reports of *Anopheles gambiae* resistance to pyrethroid in Nigeria (Djouaka et al. 2016; Omotayo et al. 2022) and Columbia (Granada et al. 2021), and highlights resistance profile with other insecticides. Mosquito susceptibility to bendiocarb was recorded compared to others. Generally, knockdown recorded in this study varied with insecticide and exposure time. Knockdown in propoxur and permethrin ranged from >20 to ≥90%, lambda-cyhalothrin and pirimiphos-methyl ranged from <10 to <90% and bendiocarb >40 to 100% from 10 to 60 minutes exposure time. Synergism of the insecticides under study, the use of synergist or appropriate estimation of the knowledge, attitude and practice of residents on insecticide use could help to monitor the possible factors responsible for insecticide resistance. High knockdown resistance mutation has been observed in some studies conducted in farm fields in western Africa (Chouaibou et al. 2012; Edi et al. 2012), and this is likely due to intermittent use of chemicals within the area. Intermittent use of chemical in the locations in this present study is no exception as suspected resistance and resistance is predicting knockdown resistance mutation. Studies on the mechanism of insecticide resistance in *Anopheles* species is ongoing and establishing an understanding in the mechanism of resistance to an insecticide is key to monitoring resistance development in the population (Karunaratne et al. 2018).

Results from the present study revealed that knockdown of 50 and 95% mosquitoes were very low, indicating the possible onset of resistant population in the area. It is probable that low knockdown in an insecticide is not proportional to resistance after 24 hours. In this present study, knockdown in pirimiphos-methyl from 10 to 60min is quite low but suspected resistance was observed after 24 hours. However, in bendiocarb high knockdown equated high susceptibility. The differences in these insecticides could be well understood from their principles of action as well as if their use has been abused in the area.

5. Conclusion

The current study clearly showed that *Anopheles* mosquitoes were highly susceptible to bendiocarb. On the contrary, mosquitoes were resistant to lambda-cyhalothrin, and suspected resistance was observed in mosquitoes exposed to permethrin and pirimiphos-methyl, which may possibly be due to their consistent use in the LGA. Considering the potentials of bendiocarb, their use in treating long lasting insecticide nets and indoor residual spray in the LGA could therefore be encouraged. Periodic insecticide monitoring is equally encouraged especially with resistant mosquitoes and those showing suspected resistance.

Author's Contribution

VN and TA conceived and designed the study. TA, CC and JU collected the field data. VN analyzed the data. J, E, TA, CC, VN and JU interpreted the analyzed data, wrote and reviewed the manuscript. All authors read and approved the final manuscript.

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